Supporting Information

Yield Improvement of the Anti-MRSA Antibiotics WAP-8294A by CRISPR/dCas9 Combined with Refactoring Self-protection Genes in *Lysobacter enzymogenes* OH11

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Table S1. Bacterial strains and plasmids used in this study

Bacterial strains/plasmids	Relevant characteristics[a]	Source/references
Lysobacterenzymogenes		
OH11	Wild-type, Km^r	26
dCas9-ω1	OH11 containing vector pB-dCas9-ω-cr1	This study
dCas9-ω2	OH11 containing vector pB-dCas9-ω-cr2	This study
dCas9-ω3	OH11 containing vector pB-dCas9-ω-cr3	This study
dCas9-ω4	OH11 containing vector pB-dCas9-ω-cr4	This study
dCas9-ω5	OH11 containing vector pB-dCas9-ω-cr5	This study
dCas9-ω6	OH11 containing vector pB-dCas9-ω-cr6	This study
dCas9-ω3/refactored	Plasmid pHmgA-P::WAP integrated into the genome of dCas9-ω3	This study
WT/refactored	Plasmid pHmgA-P::WAP integrated into the genome of OH11	This study
Other bacteria		
Bacillus subtilis	Indicator strain of WAP-8294A	3
Escherichia coli strain XL-1 Blue	Host strain for molecular cloning	Laboratory collection
Plasmids		
pBBR1-MCS5	Broad-host-range vector with P*lac* promoter, Gm^r	27
pWJ66	Plasmid contains crRNA and *dCas9*	Addgene
pB-dCas9-ω-cr1	pBBR1-MCS5 cloned with dCas9, ω subunit and cr-spacer1-cr, Gm^r	This study
pB-dCas9-ω-cr2	pBBR1-MCS5 cloned with dCas9, ω subunit and cr-spacer2-cr, Gm^r	This study
pB-dCas9-ω-cr3	pBBR1-MCS5 cloned with dCas9, ω subunit and cr-spacer3-cr, Gm^r	This study
pB-dCas9-ω-cr4	pBBR1-MCS5 cloned with dCas9, ω subunit and cr-spacer4-cr, Gm^r	This study
pB-dCas9-ω-cr5	pBBR1-MCS5 cloned with dCas9, ω subunit and cr-spacer5-cr, Gm^r	This study
pB-dCas9-ω-cr6	pBBR1-MCS5 cloned with dCas9, ω subunit and cr-spacer6-cr, Gm^r	This study
pHmgA-P	pJQ200SK containing the HmgA fragment and P_{HSAF}promoter, Gm^r	22
pHmgA-P::WAP	pHmgA-P containing the genes *orf9*, *orf10*, *orf7* and *orf6* under the P_{HSAF}promoter, Gm^r	This study

[a] Km^r, kanamycin resistant; Gm^r, gentamicin resistant

Table S2. Primers used in this study

Primers	Sequence(5’-3’)
Tra-dCas9-F-ApaI	AAAGGGCCCAAAAAAAGCACCGACTCGGTG
Tracr-Cas9-R	TACGTCGACAGCTGCGTCACCTCCTAGCTG
ωsub-F	TTAGTCGACGCCCGCATCACCGTCGAA
ωsub-R	TATCTGCAGTCAGTCGTCGCCCTTGGA
Cr-up-F	TCGCTGCAGTACTCTTAATAAATGCAGT
Cr-R-XbaI	GGCTCTAGAATCAAGCTTATCGATGGT
Spacer1-up-R	GCGCATTTCACCGAAATTTACGGGTTATGGGTTTTGGGACCATTC
Spacer2-up-R	CAAAGCGCGTCACAACCGCGATGACATTCGGTTTTGGGACCATTC
Spacer3-up-R	CGATGACATTCGTCATGCGGACGATGGTGAGTTTTGGGACCATTC
Spacer4-up-R	CGACCACCTGTCCGTCTTTCGCCATAACCCGTTTTGGGACCATTC
Spacer5-up-R	CCTGTCCGTCTTTCGCCATAACCCGTAAATGTTTTGGGACCATTC
Spacer6-up-R	TAACCCGTAAATTTCGGTGAAATGCGCCGGGTTTTGGGACCATTC
Spacer1-down-F	CCATAACCCGTAAATTTCGGTGAAATGCGCGTTTTAGAGCTATGC
Spacer2-down-F	CGAATGTCATCGCGGTTGTGACGCGCTTTGGTTTTAGAGCTATGC
Spacer3-down-F	TCACCATCGTCCGCATGACGAATGTCATCGGTTTTAGAGCTATGC
Spacer4-down-F	GGGTTATGGCGAAAGACGGACAGGTGGTCGGTTTTAGAGCTATGC
Spacer5-down-F	ATTTACGGGTTATGGCGAAAGACGGACAGGGTTTTAGAGCTATGC
Spacer6-down-F	CCGGCGCATTTCACCGAAATTTACGGGTTAGTTTTAGAGCTATGC
WAPS1-real-F	AACATCAGGCCAGCTTGTTC
WAPS1-real-R	AGGGTCTGGAAACTCGGATT
WAPS2-real-F	TACCACCTGCTCGGCTATTC
WAPS2-real-R	ATGTTCTGCAGGAAGCCTTG
ORF5-real-F	GACAACCCGGAATCATTTGT
ORF5-real-R	GTCGATCAGGTGCTTGGTCT
OH11-16S-real-F	GTGCGTAGGTGGTTTGTTAA
OH11-16S-real-R	ATCTAATCCTGTTTGCTCCC
ORF6-up	CCGCTCGAGCTCACCCGCTTCGCAA
ORF6-down	CCGCTCGAGGCATCGCATTGGCG
ORF7-up	CGCGGATCCCCATCAAGGAGGTTGACC
ORF7-down	CCGCTCGAGTCAGCCCGTGGTCAACAC
ORF9-10-up	CGCGGATCCATGTCCCGAACCGTATTG
ORF9-10-down	CGCGGATCCTCAACGGTTCAGGCCTT
ORF6-VF1	TTGGGGTAGTGAAGACGCA
ORF6-VR1	GTGTTGACCACGGGCTGA
ORF6-VF2	GCCAGGGTTTTCCCAGTC
ORF6-VR2	CCTGTTCGCACAAGCCTG
ORF6-real-F	CGCCTTCAACCTGACCCA
ORF6-real-R	GACCGCTGTCGTTGTTGG
ORF7-real-F	GTTATCGCCTGGTCGTGG
ORF7-real-R	GTCAGCGGCTTGTGCAGA
ORF9-real-F	CCTTGTTCCTGGAGCACG

ORF9-real-R	TTCGTCCAGGCGGATGTC
ORF10-real-F	CCCGATTACGAGCGCTAT
ORF10-real-R	GCCGCCACCAGTAGTTGA
Gm-F	GCAGCAACGATGTTACGC
Gm-R	CTTCCCGTATGCCCAACT

Table S3. Test of WAP-8294A2 efficacy

Organism	Reference Number	Features[a]	MIC (μg/ml)
Streptococcus pyogenes	B66-13		51.2
Streptococcus pyogenes	ATCC 12384		25.6
Streptococcus pyogenes	A-B687-12		51.2
Streptococcus pyogenes	A-W22962		25.6
Streptococcus pyogenes	A-W57924		25.6
Streptococcus agalactiae	B223-12		>51.2
Streptococcus agalactiae	B195-13		>51.2
Streptococcus agalactiae	B184-13		>51.2
Streptococcus agalactiae	ATCC 12386		>51.2
Streptococcus agalactiae	ATCC 13813		>51.2
Streptococcus pneumoniae	B343-13		>51.2
Streptococcus pneumoniae	B396-13		>51.2
Streptococcus pneumoniae	B419-13		>51.2
Streptococcus pneumoniae	B454-13		>51.2
Streptococcus pneumoniae	B500-13		>51.2
Enterococcus faecalis	B161-13		51.2
Enterococcus faecalis	B103-13		51.2
Enterococcus faecalis	B114-13		51.2
Enterococcus faecalis	B126-13		51.2
Enterococcus faecalis	ATCC 29212		51.2
Enterococcus faecium	ATCC 51559	VRE	51.2
Enterococcus faecium	ATCC 51299	VRE	51.2
Enterococcus faecium	V-18-13	VRE	25.6
Enterococcus faecium	V-19-13	VRE	25.6
Enterococcus faecium	V20-13	VRE	51.2
Enterococcus faecium	V22-13	VRE	51.2
Enterococcus faecium	E16-69	Dapto R	51.2
Enterococcus faecium	E16-70	Dapto R	51.2
Enterococcus faecium	E16-71	Dapto R	25.6
Enterococcus faecium	E17-21	Dapto R	25.6
Enterococcus faecium	E17-38	Dapto R	51.2
Staphylococcus aureus	ATCC BAA-977		0.4
Staphylococcus aureus	ATCC-43300	MRSA	0.4
Staphylococcus aureus	ATCC 25923	MSSA	0.4
Staphylococcus aureus	B163-13	MRSA	0.4
Staphylococcus aureus	B176-13	MRSA	0.2
Staphylococcus aureus	M517-12	Dapto R	0.4
Staphylococcus aureus	B214-13	Dapto R	0.8
Staphylococcus aureus	B215-13	Dapto R	0.8
Staphylococcus aureus	M305-11	Dapto R	0.8
Staphylococcus aureus	M236-11	Dapto R	0.4
Staphylococcus epidermidis	ATCC 14990		1.6

Staphylococcus epidermidis	B199-13	0.8
Staphylococcus epidermidis	B168-13	1.6
Staphylococcus epidermidis	B166-13	1.6
Staphylococcus epidermidis	B150-13	1.6
Listeria monocytogenes	NPHL-2354	3.2
Listeria monocytogenes	NPHL-5606	6.4
Listeria monocytogenes	NPHL-2326	6.4
Listeria monocytogenes	NPHL-2362	6.4
Listeria monocytogenes	NPHL-2358	6.4
Bacillus species	NPHL-6420	0.4
Bacillus cereus	NPHL-2810	>12.8
Bacillus cereus	NPHL-2990	>12.8

[a]VRE = vancomycin-resistant *Enterococci*

Dapto R = Daptomycin-resistant isolate

MRSA=Methicillin-resistant *Staphylococcus aureus*

MSSA = Methicillin-susceptible *Staphylococcus aureus*

Table S4. Statistics of the transcription level of *WAPS1*, *WAPS2* and *orf5* in dCas9-ω strains. Tukey Test was used to analyze the data.

Strains	*WAPS1*			*WAPS2*			*orf5*		
	Mean	5% SL[α]	1% SL	Mean	5% SL	1% SL	Mean	5% SL	1% SL
WT	1.0000	c[β]	B[γ]	1.0000	c[β]	C[γ]	1.0000	b[β]	B[γ]
Spacer 2	1.7270	c	B	1.9830	c	BC	4.0920	b	B
Spacer 3	5.1820	a	A	8.8037	a	A	48.8467	a	A
Spacer 4	1.4863	c	B	1.7940	c	BC	1.5723	b	B
Spacer 5	3.8323	b	A	4.0633	b	B	4.1367	b	B
Spacer 6	0.5563	c	B	0.6533	c	C	0.9297	b	B

[α]Significance Level; [β]the lowercase letters indicate the *P* value < 0.05 between WT and the other strains, when their letters are different from that of WT; [γ]the capital letters indicate the *P* value < 0.01 between WT and the other strains, when their letters are different from that of WT.

Table S5. Statistics of the transcription level of *orf9*, *orf10*, *orf7* and *orf6* in various *Lysobacter* strains. Tukey Test was used to analyze the data.

Strains	*orf9*			*orf10*			*orf7*			*orf6*		
	Mean	5% SL[α]	1% SL	Mean	5% SL	1% SL	Mean	5% SL	1% SL	Mean	5% SL	1% SL
WT 24h	1.0000	c[β]	C[γ]	1.0000	c[β]	C[γ]	1.0000	c[β]	C[γ]	1.0000	bc[β]	B[γ]
dCas9-ω3 24h	0.8283	c	C	0.5833	c	C	1.3803	c	C	0.7433	bc	B
dCas9-ω3/refactored 24h	65.7063	a	A	28.4610	a	A	20.4257	a	A	21.4303	a	A
WT 48h	4.0993	c	C	1.1160	c	C	1.7113	c	C	0.8863	c	B
dCas9-ω3 48h	3.5673	c	C	0.6007	c	C	2.2597	c	C	0.3630	c	B
dCas9-ω3/refactored 48h	44.1810	b	B	9.4087	b	B	15.5280	b	B	8.3870	b	B

[α]Significance Level; [β]the lowercase letters indicate the *P* value < 0.05 between WT and the other strains, when their letters are different from that of WT; [γ]the capital letters indicate the *P* value < 0.01 between WT and the other strains, when their letters are different from that of WT.

Table S6. Statistics of the LC-MS peak areas of WAP-8294A compounds in various *Lysobacter* strains. Tukey Test was used to analyze the data.

Strains	WAP-8294A1			WAP-8294A2			WAP-8294A4		
	Mean	5% SL[α]	1% SL	Mean	5% SL	1% SL	Mean	5% SL	1% SL
WT	1.0000	b[β]	BC[γ]	1.0000	c[β]	B[γ]	1.0000	bc[β]	BC[γ]
ΔWAPS1	0.0000	c	C	0.0000	d	C	0.0000	c	C
dCas9-ω3	1.6267	b	B	1.3433	b	B	2.4767	b	B
dCas9-ω3/ refactored	6.1167	a	A	4.3567	a	A	9.4267	a	A

[α]Significance Level; [β]the lowercase letters indicate the *P* value < 0.05 between WT and the other strains, when their letters are different from that of WT; [γ]the capital letters indicate the *P* value < 0.01 between WT and the other strains, when their letters are different from that of WT.

Table S7. Statistics of the LC-MS peak areas of WAP-8294A compounds extracted from the extracellular fractions of various *Lysobacter* strains. Tukey Test was used to analyze the data.

Strains	WAP-8294A1			WAP-8294A2			WAP-8294A4		
	Mean	5% SL[α]	1% SL	Mean	5% SL	1% SL	Mean	5% SL	1% SL
WT	1.0000	c[β]	C[γ]	1.0000	c[β]	B[γ]	1.0000	c[β]	C[γ]
dCas9-ω3	3.6367	b	B	1.6900	b	B	3.2000	b	B
dCas9-ω3/ refactored	6.1067	a	A	4.1067	a	A	6.7933	a	A
WT/ refactored	1.1067	c	C	1.2967	bc	B	1.4933	c	C

[α]Significance Level; [β]the lowercase letters indicate the *P* value < 0.05 between WT and the other strains, when their letters are different from that of WT; [γ]the capital letters indicate the *P* value < 0.01 between WT and the other strains, when their letters are different from that of WT.

Table S8. Statistics of the LC-MS peak areas of WAP-8294A compounds extracted from the intracellular fractions of various *Lysobacter* strains. Tukey Test was used to analyze the data.

Strains	WAP-8294A1			WAP-8294A2			WAP-8294A4		
	Mean	5% SL[α]	1% SL	Mean	5% SL	1% SL	Mean	5% SL	1% SL
WT	1.0000	c[β]	BC[γ]	1.0000	b[β]	B[γ]	1.0000	c[β]	C[γ]
dCas9-ω3	2.1933	b	B	1.1500	b	B	1.5033	b	B
dCas9-ω3/ refactored	3.8233	a	A	2.2400	a	A	2.9000	a	A
WT/ refactored	0.6233	c	C	0.6167	c	B	0.6500	d	C

[α]Significance Level; [β]the lowercase letters indicate the *P* value < 0.05 between WT and the other strains, when their letters are different from that of WT; [γ]the capital letters indicate the *P* value < 0.01 between WT and the other strains, when their letters are different from that of WT.

Table S9. Statistics of the extracellular/intracellular ratios of WAP-8294A compounds in various *Lysobacter* strains. Tukey Test was used to analyze the data.

Strains	WAP-8294A1			WAP-8294A2			WAP-8294A4		
	Mean	5% SL[α]	1% SL	Mean	5% SL	1% SL	Mean	5% SL	1% SL
WT	1.1200	b[β]	A[γ]	1.0133	c[β]	B[γ]	0.9967	b[β]	A[γ]
dCas9-ω3	1.8600	a	A	1.6100	b	AB	2.1433	a	A
dCas9-ω3/ refactored	1.7833	ab	A	1.8600	ab	A	2.3333	a	A
WT/ refactored	2.0000	a	A	2.1967	a	A	2.2967	a	A

[α]Significance Level; [β]the lowercase letters indicate the *P* value < 0.05 between WT and the other strains, when their letters are different from that of WT; [γ]the capital letters indicate the *P* value < 0.01 between WT and the other strains, when their letters are different from that of WT.

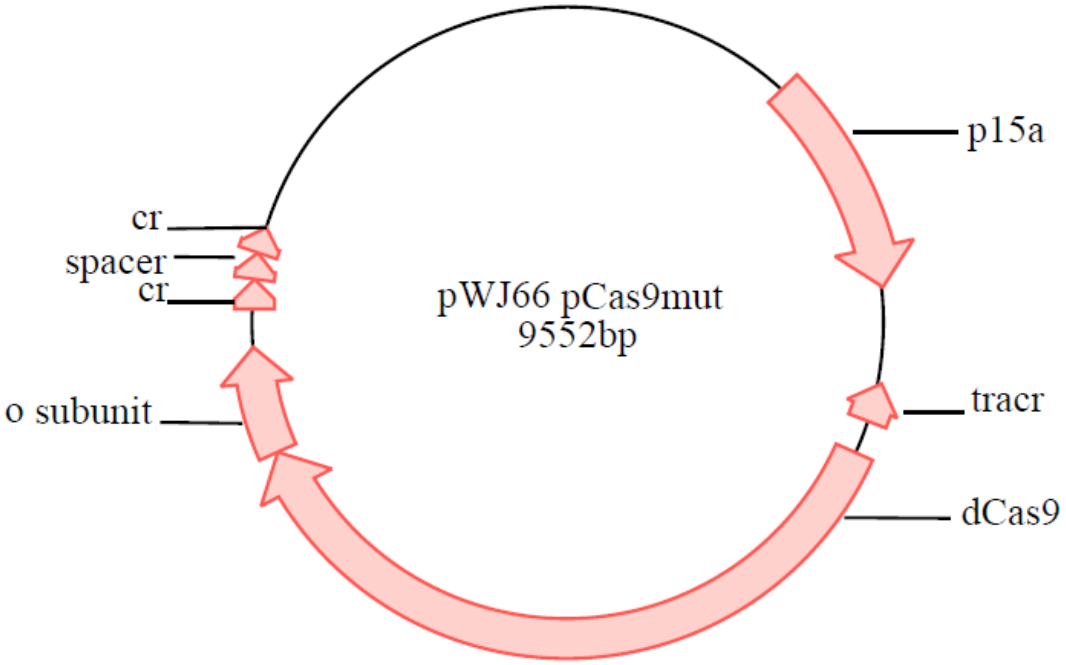
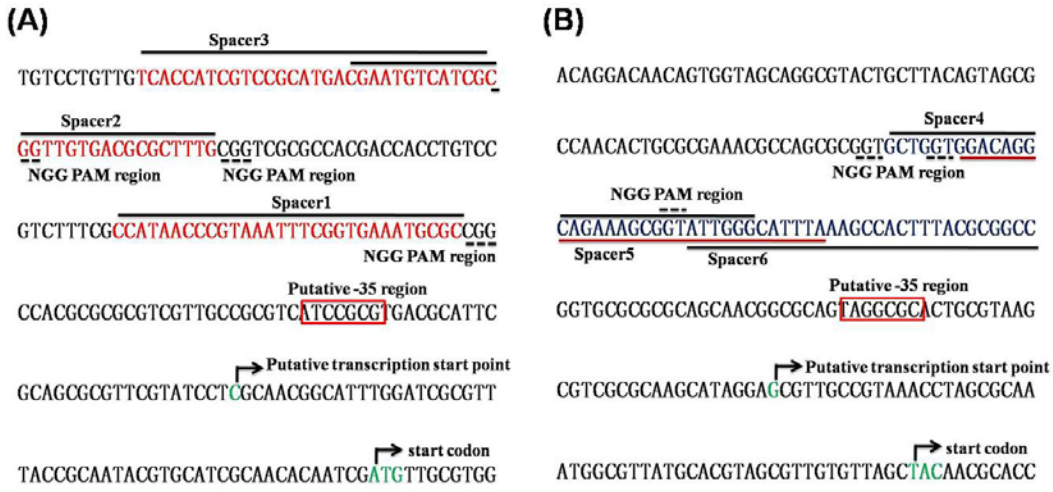


Figure S1. Map of plasmid pWJ66, which provides the elements of constructing pB-dCas9-o-cr in gene activation system CRISPR/dCas9-ω in *L. enzymogenes* OH11[17]. cr, CRISPR RNA; o subunit, omega subunit of RNA polymerase; tracr, trans-activating crRNA.



(C)

Spacer	Distance to putative -35 region
1	27
2	89
3	107
4	48
5	42
6	24

Figure S2. The position of the six spacers used in this study. The sequences are located in the upstream promoter of *orf5*. (A) The position of Spacers 1, 2, 3 in the coding strand. (B) The position of Spacers 4, 5, 6 in the non-coding strand. (C) Distance of the six spacers to the putative -35 promoter element. PAM, protospacer-adjacent motif.

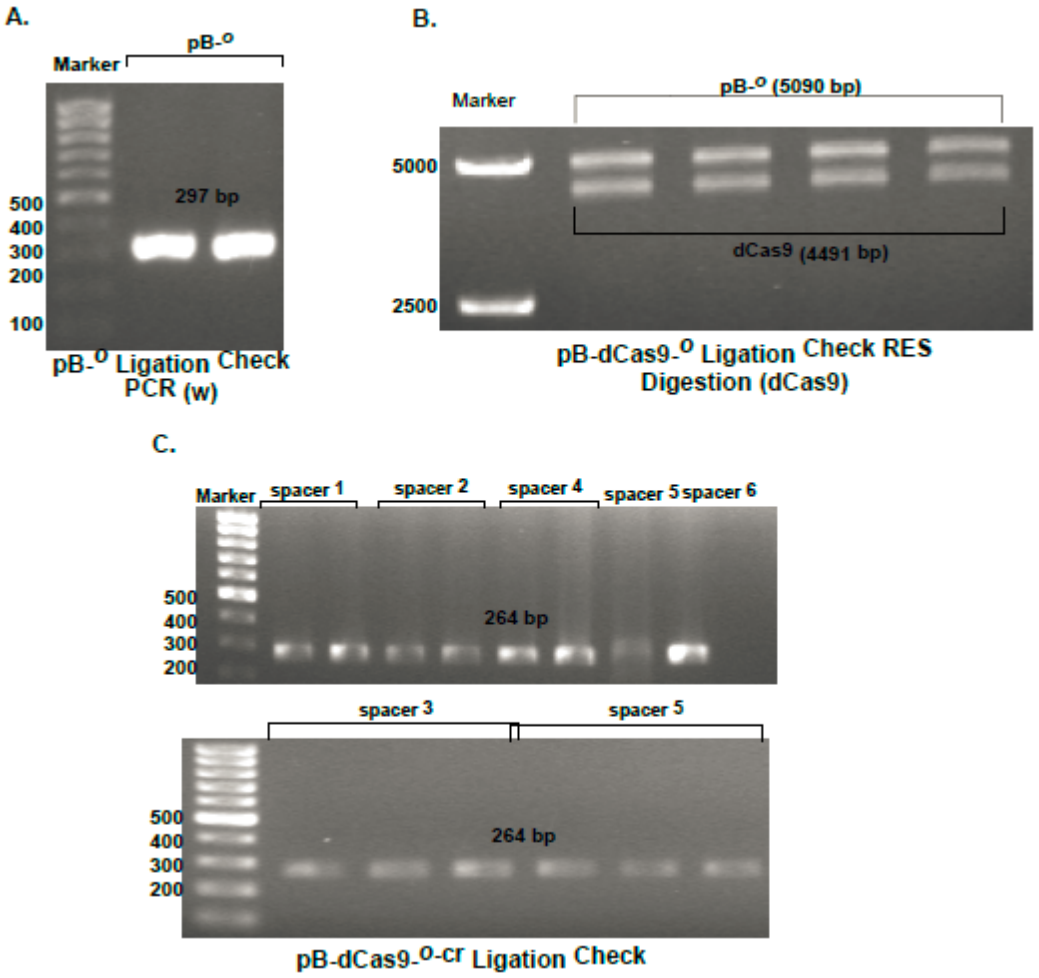


Figure S3. Confirmation of CRISPR/dCas9-ω construct for WAP gene activation in *L. enzymogenes* OH11. (A) Diagnostic PCR of ligation of omega subunit and pBBR1-MCS5 empty vector, expected 297 bp for omega subunit (ω). (B) Double digestion by *Apa*I and *Sal*I, expected 4491 bp for dCas9 gene (lower band). (C) Diagnostic PCR of pB-dCas9-*o*and six spacers ligation, expected 264 bp for crRNA (spacers).

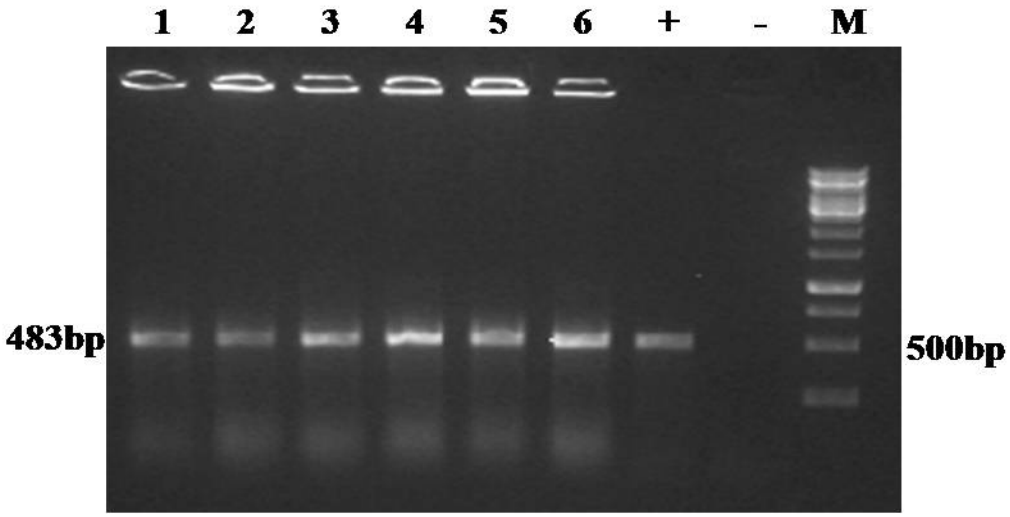


Figure S4. PCR verification of dCas9-ω strains. PCR amplification of gentamicin resistance gene using primers listed in Table S2. 1-6, *L. enzymogenes* OH11 containing each of six pB-dCas9-o-cr vectors. +, positive control, using pBBR1-MCS5 as template. -, negative control, using the gDNA of WT as template. M, 1kb DNA marker.

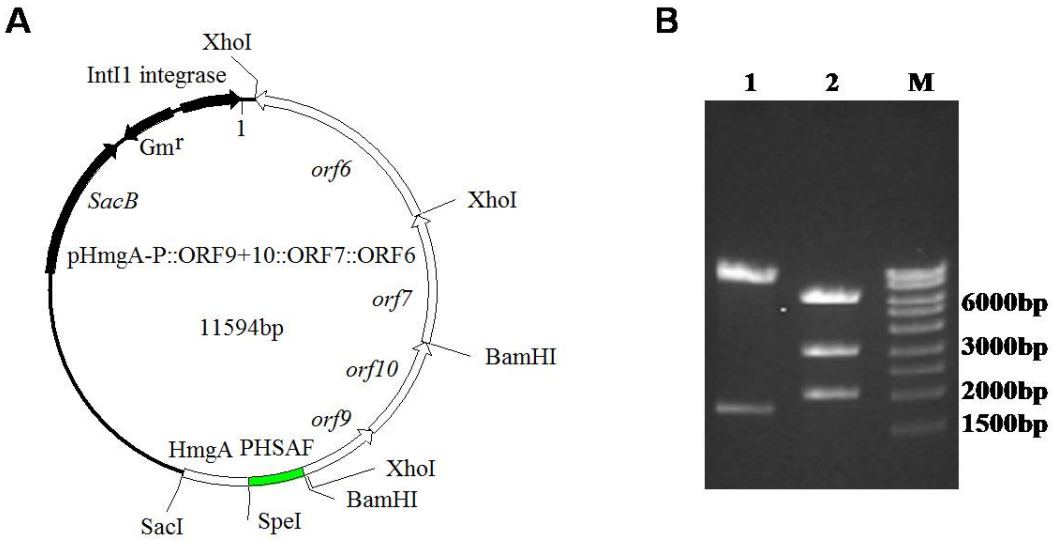


Figure S5. Verification of pHmgA-P::WAP. (A) Map of constructed plasmid containing *orf6*, *orf7*, *orf9* and *orf10*. HmgA, a part of gene sequence of *hmgA*; *SacB*, levansucrase encoding gene; Gm^r, the gentamicin resistance gene. (B) Verification of pHmgA-P::WAP. 1, plasmid treated with *Bam*HI, a product of 1780 bp was expected; 2, plasmid treated with *Xho*I, products of 3004 bp and 2018 bp were expected; M: DNA marker.

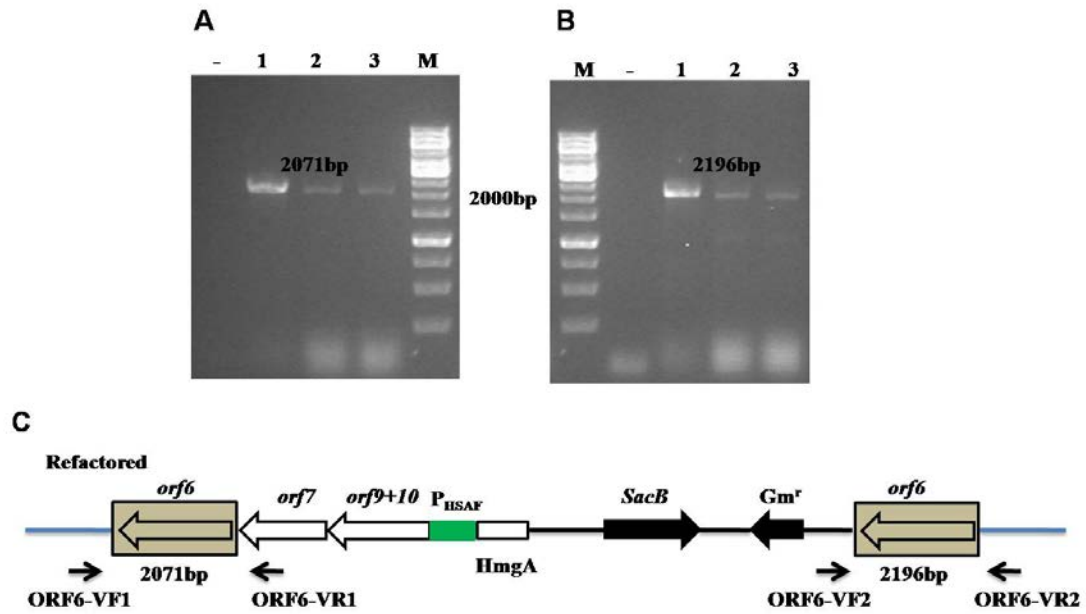


Figure S6. PCR verification of engineered strains using primers ORF6-VF1/VR1 (A) and ORF6-VF2/VR2 (B). -, negative control, gDNA of WT was used as template; 1, 2, dCas9-ω3/refactored strains; 3, WT/refactored strain; M, 1kb DNA marker. (C) The position of primers ORF6-VF1/VR1 and ORF6-VF2/VR2.

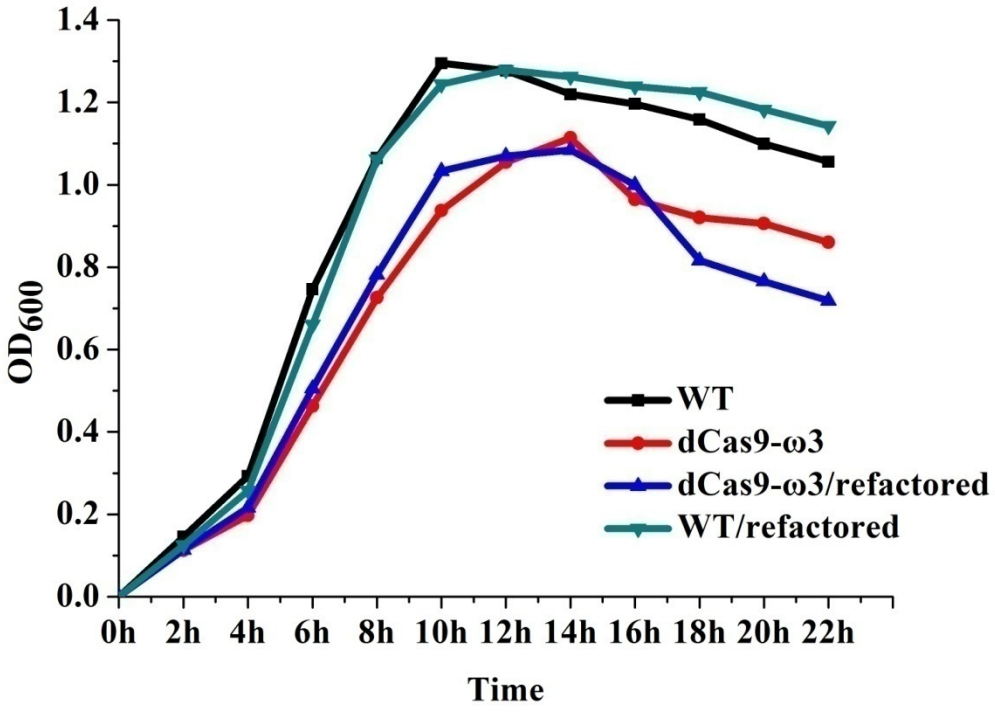


Figure S7. The growth curve of WT and engineered strains.

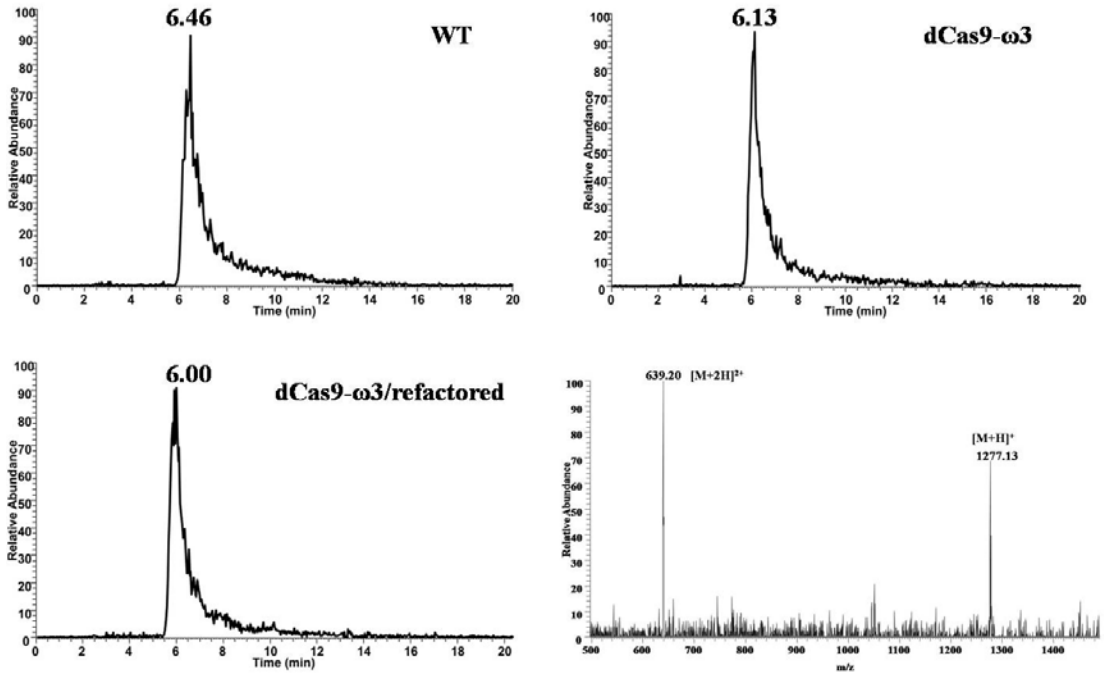


Figure S8. EIC (Extracted Ions Chromatograph) of lysobactin (as an external standard for the detection of cyclic peptides) in WT and engineered strains.

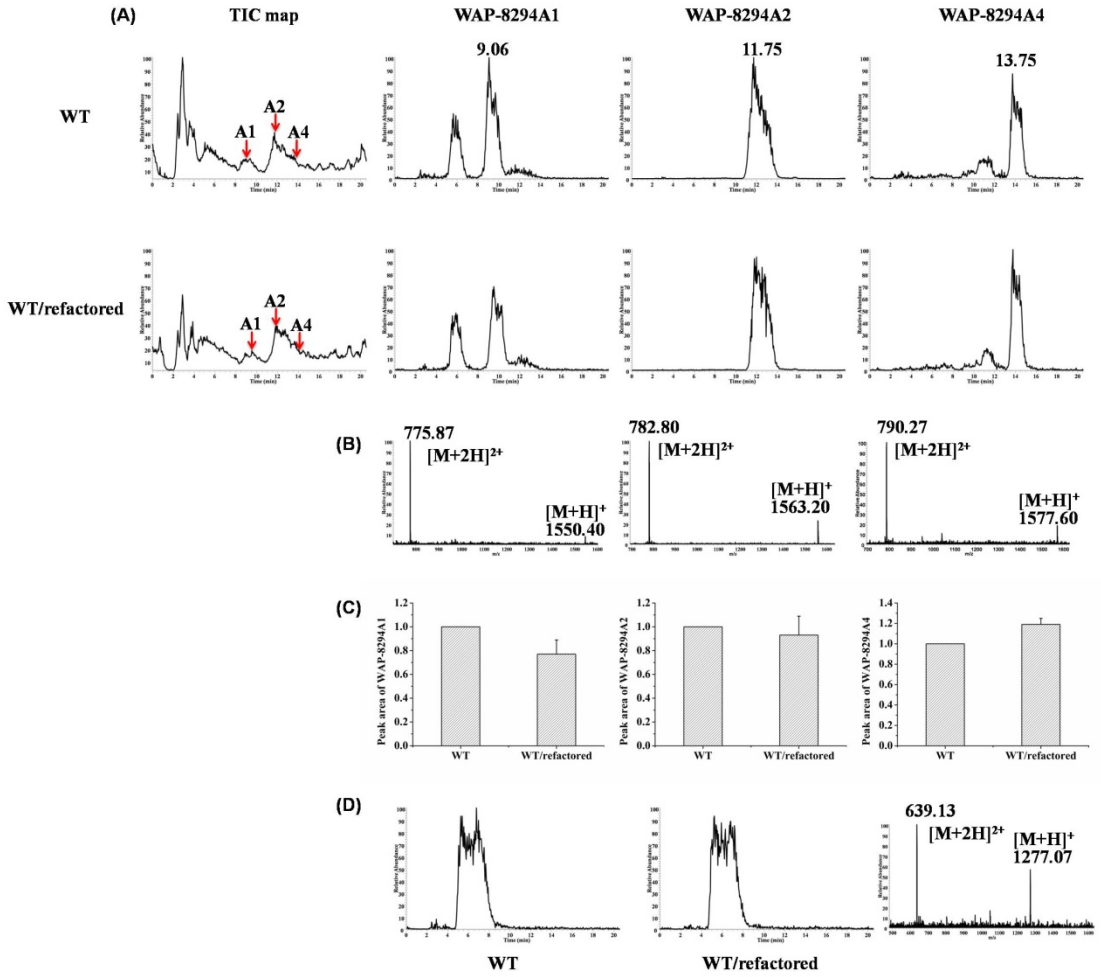


Figure S9. Quantification of WAP-8294A compounds by LC-MS. (A) Yield of WAP-8294A1, WAP-8294A2 and WAP-8294A4 in WT and WT/refactored strain. TIC, Total Ions Chromatograph. (B) The *m*/*z* of WAP-8294A1, WAP-8294A2 and WAP-8294A4. (C) Peak area of WAP-8294A compounds in WT and WT/refactored strains. (D) EIC map of lysobactin (as the external standard) in WT and WT/refactored strains.

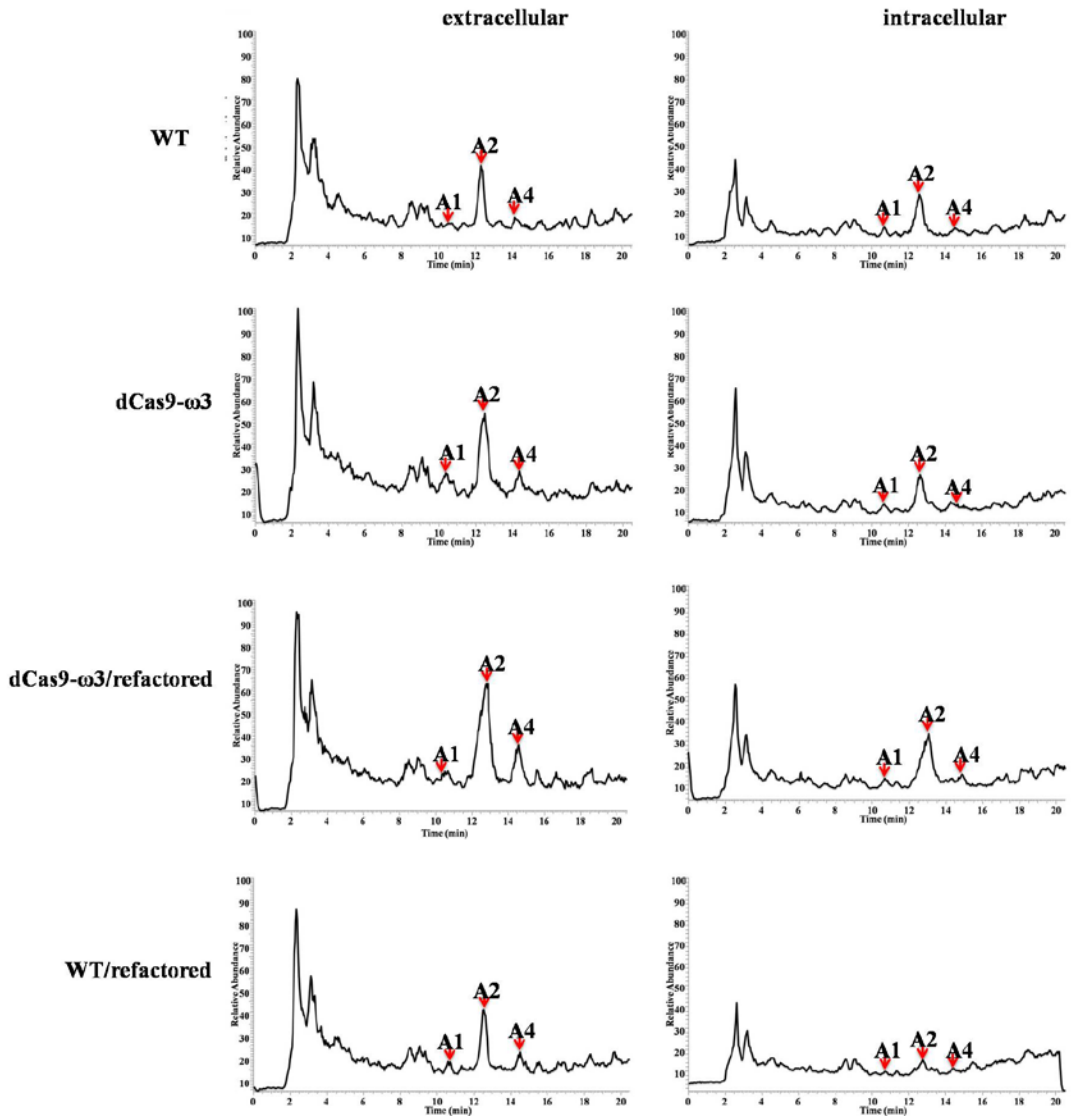


Figure S10. Total Ions Chromatograph of extracellular and intracellular WAP-8294A compounds.

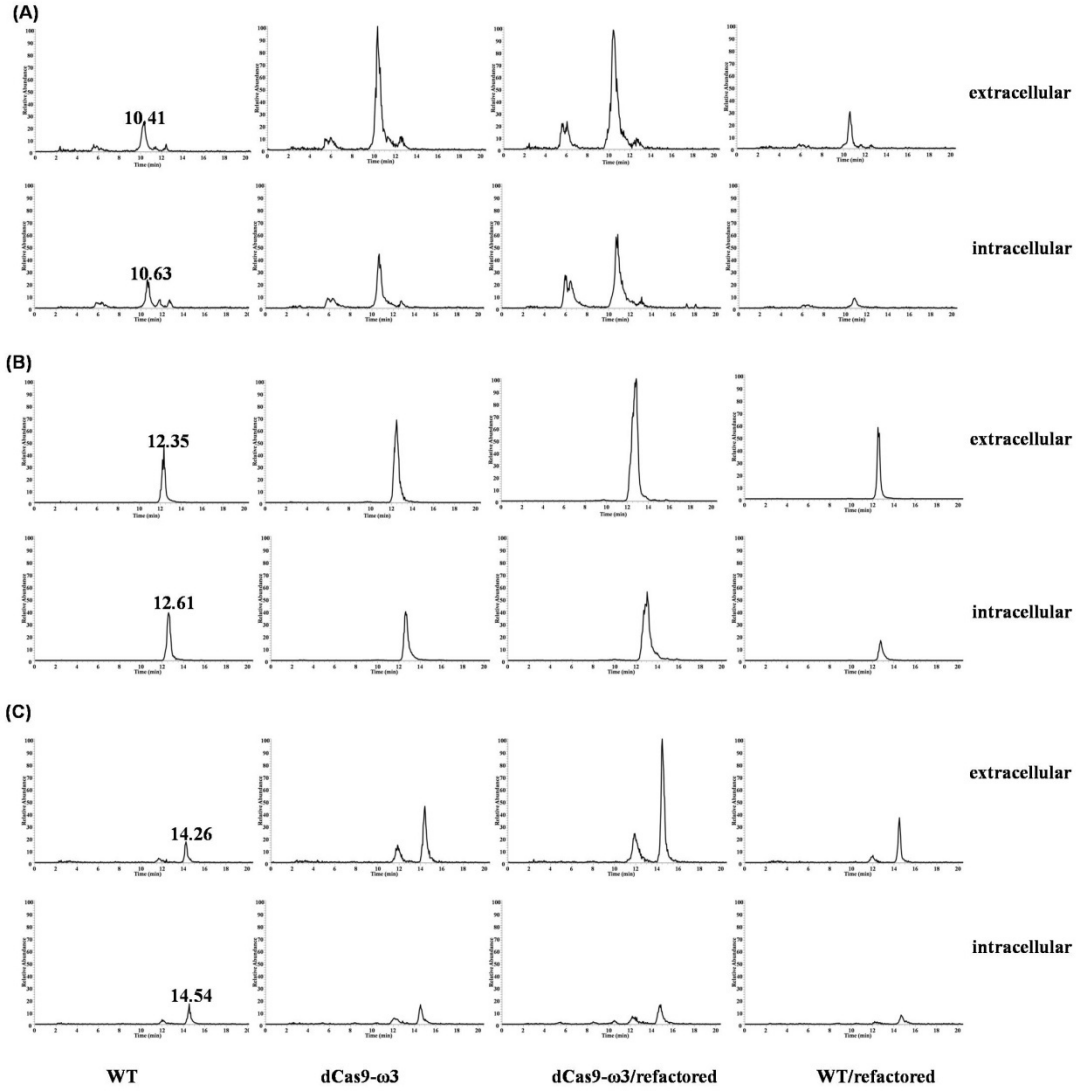


Figure S11. The distribution of WAP-8294A1 (A), WAP-8294A2 (B) and WAP-8294A4 (C) in WT and engineered strains. The upper lines represent the extracellular WAP-8294A compounds; the bottom lines represent the intracellular WAP-8294A compounds.

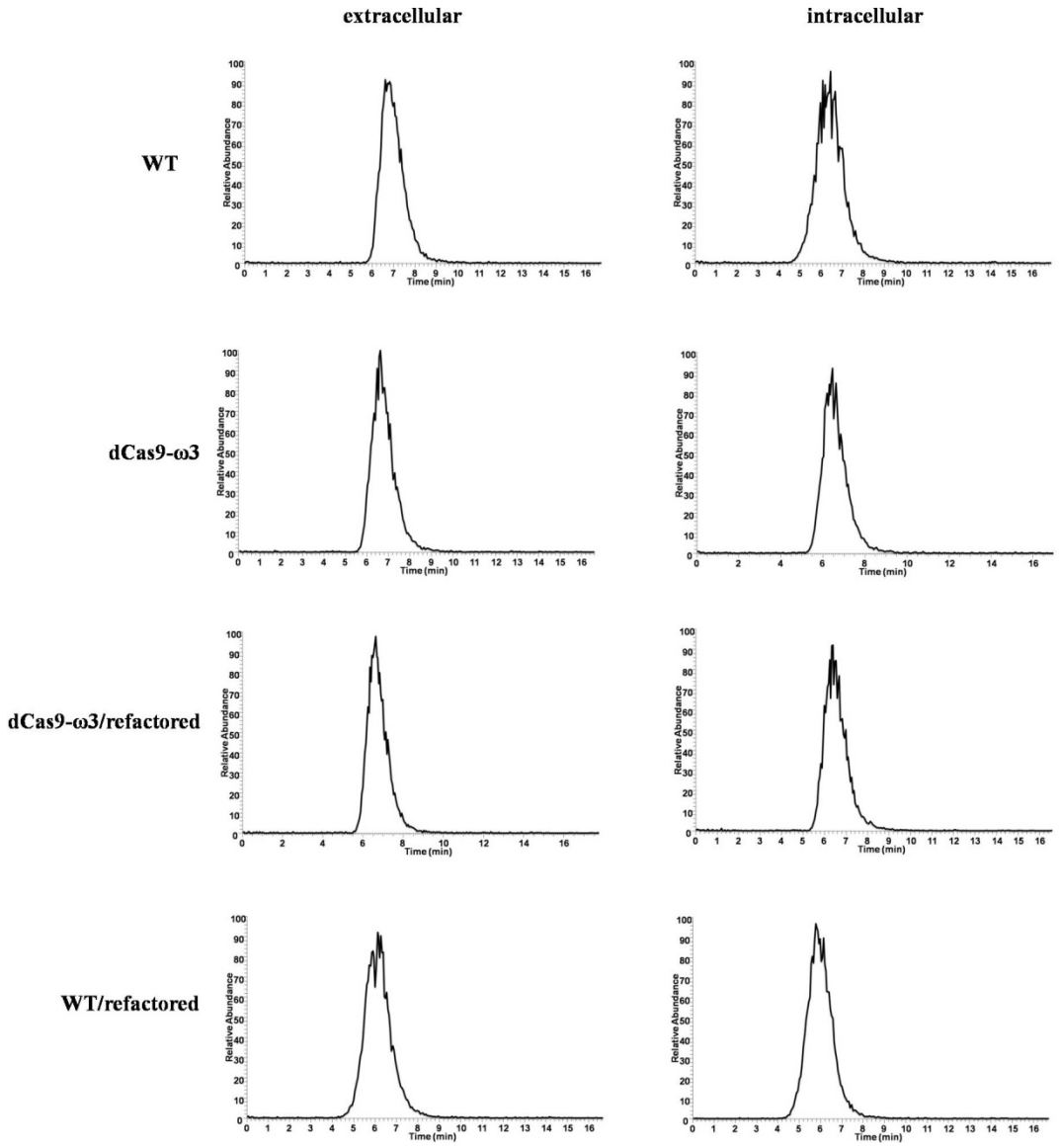


Figure S12. EIC of lysobactin (as the external standard) in WT and engineered strains.